AMERICAN SOCIETY FOR LASER MEDICINE AND SURGERY TWENTIETH ANNUAL MEETING RENO, NEVADA APRIL 7–9, 2000 ABSTRACTS

BASIC SCIENCE/ OPTICAL DIAGNOSTICS

1

A POSSIBLE ROLE OF HEAT SHOCK PROTEIN (HSP) 70 IN SELECTIVE DERMAL REMODELING

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Selective dermal remodeling using diode or Nd:YAG lasers has been recently proposed for skin rejuvenation. Thanks to this new technique, epidermis destruction is not required. The basic principle consists in moderately heating the collagen fibers since partially denaturing collagen in the dermis accelerates the collagen synthesis process by the fibroblasts. Since the basic mechanisms of this new technique is not well understood, this study aimed to evaluate (Heat Shock Protein) Hsp70 expression in normal rat skin following a moderate heating induced by a diode laser. Using a highly specific monoclonal antibody, the expression of Hsp70 along the different structures of dorsal skin from hairless rats following exposure to diode laser (λ=810nm, P=1.5W, F=145J/cm^2, $\varnothing$= 2mm) was investigated against skin without laser irradiation. In non-irradiated skin, the analysis revealed a constitutive expression of Hsp70 in the upper layer of the epidermis (stratum spinosum) and appeared only minor in the dermal papillary layer. In laser irradiated skin, very dense labeling was observed in the epidermis and in some dermal cells, particularly around blood vessels from both the papillary layer and the hypodermis. Cells around and within sebaceous glands, sweat glands or hair follicles were also densely stained. Laser-induced expression of Hsp70 was still present 7 days after laser irradiation. Since it has been already demonstrated that Hsp70 play a direct role in repairing heat-damaged cells, Hsp70 might contribute to improve wound healing and dermal remodeling.

2

NON-ABLATIVE ER:YAG LASER SKIN RESURFACING WITH REPETITIVE EXPOSURE AND CRYOGEN SPRAY COOLING

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The purpose of this study was to ascertain the feasibility of non-ablative skin resurfacing using repetitive Er:YAG laser exposure (λ = 2.94 μm) and cryogen spray cooling, using both theoretical and experimental approaches.
The stacking of up to 20 Er:YAG laser pulses (Fidelis by Fotona, Ljubljana) on the same skin site at high repetition rates (up to 50 Hz) is used to achieve substantial heat accumulation in the dermis. The energy density of individual laser pulses is kept below the ablation threshold value (0.5-1.0 J/cm^2) to prevent disruption of the epidermis. At the same time, short spurts of cryogen liquid (5-20 ms) are applied a few miliseconds before or after the 1-ms laser pulses to further reduce epidermal thermal damage. Prior to experiments, the combined effects of laser heating and cryogen spray cooling were explored using a one

*The author(s) acknowledge that proprietary disclosure is required for material presented in the abstracts denoted by an asterisk.

dimensional numerical model. Based on the results, a protocol was designed for an *in vivo* study in rats. Depth of dermal collagen coagulation as well as epidermal damage were assessed from histologic sections from laser exposed sites.
Coagulation of dermal collagen to depths in excess of 100 μm with no ablation and minimal epidermal thermal damage was observed in specimens taken one hour post-laser.
With rapid stacking of low fluence Er:YAG pulses, coagulation depths similar to those in CO_2 laser resurfacing can be achieved. The presented method offers a novel approach to non-ablative laser skin resurfacing and may allow rhytid improvement while reducing risks associated with traditional laser skin resurfacing including infection, dyspigmentation and scarring.

3

EFFECTS OF VARIOUS ATOMIZER TYPES ON CRYOGEN SPRAY COOLING

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Cryogen spray cooling (CSC) is an effective method to reduce or eliminate epidermal damage during laser treatment of hypervascular cutaneous malformations such as port wine stains (PWS). Previous CSC studies included utilization of an automobile fuel injector as an atomizer to deliver the cryogen. This study sought to determine if CSC efficiency could be increased via different atomizers. An epoxy resin model of skin with thermocouples mounted on the surface and embedded at 20, 90, 200, and 400 μm below the surface was used to measure temperature profiles in response to CSC with different atomizer types. Experiments were conducted for various spray distances and spurt durations. Mass output at the atomizer tip was measured for each type of atomizer for given spurt durations. Considerable variation in cryogen mass output (on an order of magnitude) was found with various atomizers tested. In comparison with an automobile fuel injector, a hydraulic atomizer did not produce lower internal temperatures while inducing lower cryogen film temperature on the surface for the same spurt duration. An air atomizing spray system (with no air supplied) produced lower internal temperatures (-30°C versus -24°C at 20 μm), in comparison with the fuel injector, with similar cryogen film temperature on the surface despite delivering lower cryogen mass output. Results of this study demonstrated that equivalent cryogen spurt durations produced different mass output, and the heat transfer coefficient at the surface may vary with atomizer type.

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PROTECTING EX VIVO HUMAN EPIDERMIS FROM HIGH FLUENCE PULSED DYE LASER IRRADIATION WITH CRYOGEN SPRAY COOLING

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Higher incident laser fluences than currently used in therapy (8-10J/cm^2) are expected to result in more effective treatment of port-wine stains (PWS). However, higher fluences increase the risk of epidermal damage due to absorption of light by melanin. Cryogen spray cooling offers an effective method to reduce epidermal injury during laser irradiation. The objective of this study was to determine if high laser fluences (15-30J/cm^2) could be used while still protecting the epidermis in ex vivo human skin samples. Non-PWS skin from a human cadaver was irradiated with a Candela ScleroPlus Laser (λ=585nm; pulse duration=1.5ms) under various laser fluences (8-30J/cm^2) with and without cryogen (R-134a) spray cooling (spurt durations: 40ms-250ms). Indicators of epidermal damage, assessed by H&E histological staining method, included rupture of basal keratinocytes, separation at the basal layer, and coagulation of dermal collagen in the papillary dermis. Relatively short spurt durations (40ms-100ms) protected the epidermis for laser fluences comparable to current therapeutic levels (8-15J/cm^2). However, longer spurt durations (100ms-250ms) increased the fluence threshold for epidermal damage to as much as 30J/cm^2 in these ex vivo samples.

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MEASURED TEMPERATURE PROFILES AND THERMAL MODELS OF DIODE LASERS FOR HAIR REMOVAL

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Background and Objectives: Diode lasers can be used for hair removal. The selective absorption of the light produces a thermal transient that is lethal to the hair follicle. Clinical investigations have considered pulse length and fluence, however, the zones for thermal damage have not been studied well. ***Study Design/Materials and Methods:*** We have measured the temperature as a function of depth for a diode laser (810 nm) in a piglet model. The temperature is measured with micro-thermocouples. The temperature is recorded as a function of fluence and time. Models of light scattering and thermal diffusion are used to simulate the measured system. Histology is used to confirm the volume of thermally damaged tissue. ***Results:*** Maximum thermal energy is deposited from 1.5 to 2.0 mm below the surface. High peak intensities and short pulses limit the undesirable lateral thermal damage. The scattering of the incident beam significantly reduces the effective fluence at these depths. Excellent agreement is seen between the calculations and the measurements. ***Conclusions:*** The diode laser with sufficient peak intensities can be used for hair removal. The model provides the ability to compare different laser systems and predict the laser--tissue interactions from changes in the delivery parameters.

6

PHASE-SENSITIVE THERMAL WAVE IMAGING OF SUBSURFACE CHROMOPHORES IN BIOMATERIALS

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To improve imaging of light absorbing chromophores in tissue, we apply a thermal wave radiometry technique with amplitude and phase sensitive detection. In this technique, thermal wave is generated in a test sample by periodically modulated laser source and used for noncontact imaging

of subsurface chromophores. Infrared emission images of the periodically heated specimen and narrow band, lock-in signal processingwere utilized to compute a time-sequence of images corresponding to signal amplitude and phase. We demonstrate that phase-sensitive thermal wave imaging may be used to assess depth and dimensions of subsurface chromophores in tissue. Measurements are reported using in-vitro and in-vivo models.

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EFFECTS OF TISSUE OPTICAL CLEARING AGENTS ON FLUORESCENCE SIGNAL FROM A SUBSURFACE TARGET

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This study investigated the effects of osmotically active chemical agents on turbid tissue by monitoring fluorescence emission spectra of various subsurface fluorophores. Hyperosmotic agents, such as glycerol, DMSO, and mannitol have been shown to markedly reduce light scattering in tissue. The agents could allow deeper penetration of excitation light in the 300-500 nm range where the optical penetration depth is quite limited and can be used to investigate the effects of scattering on emission line spectra. This may allow improved interpretation of emission spectra from turbid tissue.

Hyperosmotic agents were investigated for their ability to enhance the fluorescent signal originating in a target approximately 1.5 mm below the surface of *in vitro* rodent skin. In addition, the agents were used to investigate the effect of changes in scattering properties of tissue on the line spectra of a fluorophore placed under the skin. The fluorescent targets consisted of fluorescent probes embedded in a gelatin mold. Experiments were conducted at two excitation wavelengths, 365 nm and 540 nm, corresponding to the absorption bands of the two fluorophores used – Blue FluoSpheres and Rhodamine B. Chemical agents were applied to the skin for 5, 10, and 15 minutes, and the change in signal strength and line shape were monitored over time.

Application of the agents to skin caused a reduction in light scattering. A relative increase in emission signal strength was observed within minutes of agent application. The hyperosmotic agents offer the ability to better detect the fluorescent signal from a subsurface target. Use of agents that alter tissue optical properties may lead to improved diagnostic or imaging procedures in applications such as confocal microscopy or optical coherence tomography.

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HIGH SPEED PHASE RESOLVED OPTICAL DOPPLER TOMOGRAPHY FOR IMAGING *IN VIVO* BLOOD FLOW IN HUMAN SKIN AND MONITORING LASER TREATMENT OF PORT WINE STAIN

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We have developed a novel phase resolved optical coherence (OCT) and optical Doppler tomographic (ODT) system that uses phase information derived from Hilbert transformation to image *in vivo* blood flow in human skin with fast scanning speed and high velocity sensitivity. This technique decouples the spatial and velocity resolution in flow images and increases imaging speed by more than two orders of magnitude without compromising velocity sensitivity and spatial resolution. The minimum flow velocity that can be detected is as low as 10 μm/s while maintaining a spatial resolution of 10 μm. Using this technique, the structure and distribution of blood vessels in port wine stain human skin can be imaged up to 1 mm below the surface. The effect of different laser treatment parameters, such as light dosage can be monitored using this technique.

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INFRARED SPECTROSCOPY AS NONINVASIVE *IN VIVO* DIAGNOSTIC TOOL

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Noninvasive in vivo diagnostics of living tissue and body fluids has been developed for malignancy and diseases detection in medical offices, clinics, and field hospitals.

Fourier transform infrared fiberoptic evanescent wave (FTIR-FEW) spectroscopy has been suggested as a new powerful tool and highly sensitive technique for biomedical (including clinical) diagnostics of normal, precancerous and cancerous tissue in the middle infrared (IR) region of the spectrum from 850-4000 cm^{-1} (12-2,5μm). This optical spectroscopy method is suitable for noninvasive and direct spectral measurements of normal and pathological tissue *in vivo, ex vivo* and *in vitro*. For the first time this method has been used nondestructively, rapidly (15 sec) and remotely in the operating rooms of clinics. In the review the recent new experimental results of skin, breast, lung, stomach, kidney tissue in vivo and ex vivo (during surgery) have been obtained. The method allows detecting any functional chemical group and bond, DNA bases at the molecular level directly from surfaces including living tissue. The measurements of layer structure of tissue are done the online while testing various tumor tissues during surgery to optimize and localize an area to remove tissue. The spectral studies of small spots (about 1mm in diameter) of normal and malignant tissue *in vivo* have been fulfilled with special fiber optic changeable tip probe configuration for nondestructive and noninvasive biopsy applications. The FTIR-FEW technique is also well suited for the detection of the influence of environmental factors on the skin surfaces. The techniques are also very promising for fast molecular histological examinations. In particular, this new nondestructive, fast, compact, portable, remote, and highly sensitive diagnostics tool is very promising for surface analysis at the molecular level without sample preparation. The FTIR-FEW system could be used in small and cost effective field hospitals.

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THE USE OF CONFOCAL LASER SCANNING MICROSCOPY IN MOHS MICROGRAPHIC SURGERY

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Confocal laser scanning microscopy (CLSM) is a noninvasive technique used to image biologic tissue both *in vivo* and *in vitro*. This study was designed to evaluate the accuracy and usefulness of using CLSM to image the border of a cutaneous malignancy during Mohs micrographic surgery (MMS).

Twenty patients between the ages of 20 and 80 undergoing MMS were enrolled in the study. After the tissue had been surgically excised using the MMS technique, it was scanned with the CLSM to

evaluate the borders for malignancy. A map outlining the positive margins was constructed, and the tissue was then examined with the traditional frozen hematoxylin and eosin (H&E) staining technique. The H&E stained slides were then read by the Mohs surgeon and a map was constructed outlining the positive margins. The two maps were then compared for any differences.
Although there was not 100% correlation, the maps made using confocal laser scanning microscopy correlated very well with those made using traditional H&E staining technique. This study demonstrated the usefulness of using CLSM in Mohs micrographic surgery in certain types of tumors, and highlighted the diagnostic features of CLSM images. With more powerful confocal systems, in vivo noninvasive imaging will aid in both histologic diagnosis as well as guidance of surgical treatment.

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Regional differences in the secretion of growth factors by facial and chest fibroblasts utilizing blended CO_2 and Er:YAG laser energy: a serum-free study.
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A serum-free in vitro model was used to evaluate the effect of blended CO_2 and Er:YAG laser energy upon growth factor secretion by normal human facial and chest fibroblasts. Transforming growth factor-beta 1 (TGF-β1) and basic fibroblast growth factor (bFGF) were analyzed as they are key mediators of wound healing and resultant scar quality. TGF-β1 is associated with tissue fibrosis, while bFGF promotes organized collagen bundles. Fibroblast cell lines were established from operative specimens utilizing standard explant techniques. During passage seven, cells were seeded onto 24-well trays at a concentration of 2×10^4 cells/ml in serum-free media (Ultraculture, Biowhitaker). At 48 hours, 20% of each well was exposed to 1.7 J/pulse Er:YAG laser energy and CO_2 delivered at either 3 or 5 watts, and at a duty cycle of 25%, 50% or 100%. At time = 0, 24, 72, 96 and 120 hours after treatment, cell counts and viability were measured. TGF-β1 and bFGF were assayed from collected supernatants utilizing an ELISA assay. Non-treated facial fibroblasts demonstrated statistically significantly shorter population doubling times (PDT) than normal chest fibroblasts ($p<0.01$). Laser irradiated facial and chest fibroblasts both secreted increased levels of bFGF compared with the non-laser irradiated fibroblasts. Lasered facial fibroblasts secreted less TGF-β1 than non-lasered facial fibroblasts. A trend was seen in the 3 watts 100% duty cycle group ($p<0.09$). Lasered chest fibroblasts secreted more TGF-β1 than non-lasered chest fibroblasts. This was statistically significant at 3 watts 25% and 50% duty cycles ($p<0.02$ and 0.03, respectively). The combined CO_2 and Er:YAG laser is gaining popularity as a tool for skin resurfacing, and understanding its effects upon wound healing is essential. This study shows that the blended CO_2 and Er:YAG laser decreases the production of TGF-β1 in normal facial fibroblasts. This may suggest a preventative role in excessive scar formation with facial laser skin resurfacing. On the contrary, this laser increases the production of TGF-β1 and decreases the secretion of bFGF in normal chest fibroblasts. At 3 watts 25 % or 50% duty cycles, the laser may induce excessive collagen production by normal chest fibroblasts. In addition to the differing density of adnexal structures, this growth factor secretion profile may be a component in the high incidence of scarring seen with neck/chest CO_2 – based skin resurfacing.

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EFFECT OF TEMPERATURE ON 2.1 μm Ho:YAG LASER INDUCED COAGULATION DEPTH IN NEURAL TISSUES
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When assessing tissue and vascular lesions caused by thermal lasers, it is important to consider the depth of coagulation of the cauterized tissue. The minimal thickness of tissue damage from laser coagulation should be determined in order to produce clinically optimal results. At a given laser wavelength and irradiation, the thermal response of the tissue depends on its optical properties namely, absorption and scattering, and the coagulation depth varies with temperature. In this investigation, we have improved McKenzie's three zone model (carbonized, vacuolated, and sub-boiled zones) by incorporating the scattering effects, temperature dependence of absorption and scattering, and the thermodynamical parameters of the tissue to quantify the thermal damage in neural tissues induced by a Ho:YAG laser at 2.1 μm. Our calculated depths of coagulation are 615 μm and 712 μm at 37°C (body temperature) and 100°C, respectively and increases linearly with temperature. Our calculated depth of coagulation values are in reasonable agreement with the histologically predicted thermal injury zone which extends from 400-1000 μm. Thus, our model can predict the laser induced thermal injury more accurately.

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EFFECTS OF THERMAL DAMAGE ON INFRARED OPTICAL PROPERTIES OF SKIN
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Knowledge of optical properties is important for predicting the interaction of laser light with tissue. During laser treatment, thermally-induced alterations in the tissue ultrastructure can lead to significant changes in local optical properties. Previous studies have focused primarily on dynamics in absorption and reduced scattering coefficients induced by relatively slow heating (on the order of seconds) of tissue.

To ascertain changes in optical properties caused by fast heating (μs to ms time scale) we investigated the changes in infrared optical properties of *in vitro* guinea pig skin due to laser-induced thermal denaturation. Single sites were irradiated with a variable number of laser pulses emitted from a long-pulsed (τ_p = 250 μs) Nd:YAG (λ = 1.064 μm) or Ho:YAG (λ = 2.1 μm) laser. The samples were subsequently mounted on a fast acoustic transducer (rise time = 3.5 ns) and irradiated with Q-switched (τ_p = 500 ns) Nd:YAG or Ho:YAG laser pulses. This optoacoustic setup provided a nondestructive means for monitoring changes in the local absorption and reduced scattering coefficients at 1.064 and 2.1 μm.

BIOSTIMULATION

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LOW POWER LASER TREATMENT OF CUTANEOUS WOUNDS IN *PSAMMOMYS OBESUS*
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The ability of low power laser irradiation to improve wound healing has been investigated for a number of years. This study compares different parameters of laser irradiation for their ability to improve the histological characteristics of wound healing in the Fat Sand Rat (*Psammomys obesus*), a model of Type II diabetes. These characteristics include re-epithelialization of the wound, fibroblast infiltration, neovascularization, and collagen production. Bilateral 8mm full thickness circular wounds were made on the skin of the lumbar region of the back of control and experimental non-diabetic Fat Sand Rats. Two sets of parameters using a 632.8nm HeNe laser were tested on non-diabetic animals to optimize the treatment for the diabetic Fat Sand Rats. The first set of parameters has been successfully used by Yu et al.[1] on genetically diabetic mice and involved 4 days of treatment for 250 seconds at

20mW for a total of 5J/cm²/day. The second set of parameters was tested to determine the effect of increasing the power density to 7J/cm² by increasing the treatment time to 360 seconds/day. Only the left wound of each treatment animal was irradiated, and control animals were handled identically while receiving no irradiation. At 10 days post-injury, the animals were euthanized and the wound tissue was removed, post-fixed, and sectioned. Sections were then stained with hematoxylin and eosin for histological analysis based upon the scoring scale developed by Greenhalgh et al.[2]. Histological scoring showed that control animals at 10 days had an average left wound score of 7.25 and an average right wound score of 6.70. Using Yu et al.'s parameters, we found a non-significant increase in score in both the left and right wounds of irradiated animals over the controls, indicating a systemic effect of the low power laser irradiation. The average left wound score of this group was 8.83; the average right wound score was 8.00. Using the second set of parameters, the average left wound score was 5.00, and the average right wound score was 6.00. This result suggests that these parameters are systemically inhibitory to wound healing. This study demonstrates that the establishment of optimal parameters for low power laser treatment is essential, as changes in animal models and laser parameters can have important consequences on the treatment outcome. Optimization of the laser parameters is continuing before testing begins on wounds of diabetic Fat Sand Rats.

(1)LSM 20:56-63 (1997); (2)Am J Path 136:1235-1246 (1990)

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LOW LEVEL LASER THERAPY PROMOTES WOUND HEALING EFFECT USING ARGON, KRYPTON, AND CO_2 LASERS

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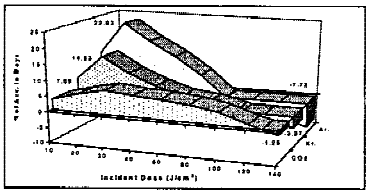
An experiment was undertaken to observe the effects of wound healing on rats using Argon, Krypton, and Carbon dioxide lasers. Sprague-Dawley rats were utilized. An oval skin wound was created aseptically with a scalpel on the shaved back of the animal after anesthesia. All animals with wounds were divided randomly into treatment and control groups. The laser beam was aligned to cover the entire wound area including the boundaries. The control group also received the same manipulation, excluding the laser exposure. The laser output power were 100, 150 and 300 mW as well as laser power density were 31.85, 35.46 and 66.37 mW/cm² in the Argon laser, Krypton laser, and Carbon dioxide laser, respectively. The incident dose was used from 10 J/cm² up to 140 J/cm² in all lasers. Our results showed that the percentage of wound healing acceleration in days were 22.93% at the optimal stimulative dose of 20 J/cm² (actual dose of 5.21 J/cm²) in Argon laser, 14.53% at the optimal stimulative dose of 20 J/cm² (actual dose of 4.03 J/cm²) in Krypton laser and 7.69% at the optimal stimulative dose of 30 J/cm² (actual dose of 5.25 J/cm²) in Carbon dioxide laser. Zero bioactivation shown at the incident dose of 80 J/cm² in Argon laser, 100 J/cm² in Krypton and 130 J/cm² in Carbon dioxide laser. The percentage of wound healing inhibition in days were -7.72% in Argon laser, -3.37% in Krypton and -1.25% in carbon dioxide laser at the maximal inhibitory dose of 140 J/cm². The study indicated that low-level laser therapy at appropriate dosimetric parameters could promote wound healing in rats. The acceleration effects of wound healing were dependent on the dose and wavelength used. Argon laser with 488-514 nm was the most effective in accelerating the wound healing in the laser wavelengths used in this study. Carbon dioxide laser with 10,600 nm was lesser than the visible lasers in the biostimulation process. Our study suggested also the excess of laser exposure time will delay the wound healing on rats.



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THE INFLUENCE OF LLLT ON THE REGENERATION OF THE LYMPHATIC SYSTEM, THE SKIN, MUSCLE AND CARTILAGE TISSUE.

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Purpose

This study summarizes the results of research about the regeneration of tissue under the influence of Low Laser Therapy.

Methods

Skin regeneration was investigated in vitro while muscle, cartilage and lymphatic regeneration was investigated in vivo.

The studies were animal studies and the techniques applied were 1) histological examinations in comparison with control groups ; 2) transillumination microscopy in vivo. For each study the number of, mice are N = 150 while each control group consisted of N = 50.

Results

For each tissue our studies showed a clear acceleration of the regeneration process confirmed by statistical analysis. An iconography will be shown of the histological slides as well as the microscopy images in vivo.

Conclusions

It is our believe that our study proves the positive effect of LLLT on the regeneration process and confirms the clinical results in this area.

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830NM IRRADIATION INCREASES WOUND TENSILE STRENGTH IN A DIABETIC MURINE MODEL.

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A normal healthy host rarely has a problem with wound healing, yet patients with diabetes often experience severely impaired wound healing. The purpose of this study was to investigate the beneficial effects of LLIR on wound healing, and to develop alternate therapeutic modalities to treat impaired wound healing in diabetics.

Female C57BL/Ksj/db/db genetically diabetic mice were each wounded twice on the dorsal surface with 1 cm full-thickness cutaneous incisions.LLIR was applied with an 830nm LaserMax diode laser at a power of 100mW for 1 min per wound. Treatment groups consisted of animals irradiated on days 0-4 days post-op, animals irradiated on days 3-7 post-op, and a non-treated group. All animals were sacrificed 21 days post-op, and the wound and surrounding tissue were excised.Tensile strengths were measured using a Burleigh tensiometer, and were standardized by dividing the tension of the tissue samples by their areas. This pilot study indicated that irradiation at 830nm significantly increases the tensile strength of wounds in diabetic mice. Non-treated mice had an average wound tensile strength of 1.913 g/mm², while in the treated group the average was 3.478 g/mm². Statistical analysis was completed with a Student's t-test ($p=0.0155$, $df=5$, t crit.$=2.57$, $t=3.598$). These results will be tested on the cellular level by measuring collagen matrix formation and expression of growth factors. A mirror pilot study was completed using non-diabetic BALB-c mice, and no difference was observed between treated and non-treated groups.

830nm LLIR seems to significantly enhance tissue reformation after wounding in a diabetic model. Despite the lack of a well-defined mechanism of the action, LLIR therapy may represent an important alternate treatment in management of diabetic wounds. Further investigation of the precise mechanism of LLIR is warranted.

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THE INFLUENCE OF IR-LASER, ON THE PROLIFERATION OF FIBROBLASTS: AN IN-VITRO STUDY

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Purpose:
In contribution on the effects of the positive influence of LLLT on the mechanism of wound healing, we investigaded whether IR-Laser (GaAs, 904 nm, pulsated) has an influence on the proliferation of fibroblasts, wich are responsable for the necessary wound healing.

Methods:
To control the reproductability, the inter-and intra reliability, we cultivated cells coming from the abdomen of two different (NMRI) mice and we divided 4 groups per mouse. Two were irradiated, two were not. Then we did a BrdU-labeling with 4 flasks (2 were irradiated, 2 were controlled). Differences between the experimental and control groups were examined a t-test and a non parametric Mann-Whitney test

Results:
Our results show a significant ($p<0,05$) increase of fibroblasts proliferation after IR-irradiation. The BrdU-labeling showed an increased DNA activity. There is also a perfect match between the increased number of fibroblasts and the DNA activity.

Conclusion
IR-low level laser therapy has a positive influence on fibroblast proliferation and will enhance the wound healing process

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MEMBRANE MECHANISM OF LOW INTENSITY LASER BIOMODULATION ON A CELL

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On low intensity laser biomodulation on a cell, Karu TI et al have suggested its mitochondria mechanism (MM) by means of the effective resonant interaction of photons with the terminal respiratory chain oxidases or flavoproteins, and Liu TCY et al have suggested its signal transduction mechanism (STM). In this paper, we study how the membrane mediates STM in terms of time quantum theory of radiation-matter interaction. In our opinion, it is the membrane receptors that mediate STM. As the frequency of the absorption light of membrane receptors is greater than the one of visible laser irradiation, the membrane absorption of visible light is non-resonant, and its transition rate is extraordinarily small, but can be amplified by the coherent state of the identical and independent membrane receptors. It is shown that the greater the frequency of a laser irradiation is, the more effective it is. The localized factors such as chemical molecules or resonant light-receptor interaction will inhibit the activation of STM by destroying the coherent state. The successful applications show that membrane-receptor-mediated STM is one of the possible mechanisms of low intensity laser biomodulation.

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LOW INTENSITY MONOCHROMATIC INFRARED THERAPY IN THE TREATMENT OF LATERAL EPICONDYLITIS: PRIMINLARY FINDINGS

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Lateral epicondylitis (LE) is a troublesome disorder of the arm causing prolonged disability, sick leave and (in certain jobs) early retirement; it is the result of repeated microtrauma that exceeds the tissues' ability to adapt. Low intensity laser therapy is a popular non-invasive treatment, which is used to stimulate tissue healing and alleviate pain[1]. However, despite its popularity, use of infrared therapy remains contentious because of the lack of definitive evidence of clinical efficacy. To address this, the current study was designed to establish the efficacy of monochromatic infrared therapy in the management of LE in a controlled and objective manner. Following ethical approval from the University of Ulster's Research Ethical Committee, male and female subjects aged between 18-80 years with LE of less than six months duration were recruited from industry, tennis clubs, fitness centres, local GP practices and the university population. Informed written consent was obtained and subjects were randomly allocated to one of two trial conditions: *Active Treatment (n=5);* GaAlAs (840nm) monochromatic infrared irradiation was applied over the area of the lateral epicondyle (840nm; pulsed 57Hz; 15.5J/cm^2; BioFlex Professional, Meditech Int. Toronto, Canada). *Sham Treatment (n=5);* subjects were treated identically to the active group, however, no active irradiation was delivered by the apparatus. Subjects were treated 3 times a week for 4 weeks and assessed at first and last attendance and three months following discharge. Outcome measures of pain and function were taken as follows: Patient Rated Forearm Evaluation Questionnaire[2], pain at rest and on palpation marked on a printed Visual Analogue Scale (VAS), maximal pain-free wrist extension force (N) maximal pain-free grip strength and, maximal grip strength tested in a standardised patient position using a Martin vigroimeter. Preliminary analysis of data indicates improvements in all outcome measures in both the active and sham treatment groups. The encouraging results of the current preliminary study would suggest that LIMIRT may offer some benefit in the treatment of LE; further research is now warranted.

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DELAYED ONSET MUSCLE SORENESS- A PRELIMINARY ASSESSMENT OF THE EFFECT OF LOW INTENSITY MONOCHROMATIC INFRARED THERAPY

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Delayed onset muscle soreness (DOMS) is a common phenomenon associated with eccentric or novel exercise[1]. The current study for which ethical approval was obtained sought to assess the effect of low intensity monochromatic infrared therapy (LIMIRT) on experimentally induced DOMS. Volunteers were recruited (n=24, 12M:12F), screened for any relevant pathology, current arm pain, use of medication, diabetes and regular weight training, and were randomly allocated under strict double blind conditions to one of three experimental groups (n=8. 4M:4F): Control, Placebo and Laser treatment groups (840nm; 1.4Jcm^{-2} at a pulse frequency of 1 kHz; Bioflex Professional Model, Meditech, Toronto). DOMS was induced in the biceps brachii of the non-dominant arm using one exhaustive bout of eccentric exercise with a weight equal to the concentric one repetition maximum. Subjects attended on five consecutive days and visual analogue scales (VAS), isometric peak torque (IPT), mechanical pain threshold (MPT) and resting angle (RANG) were used to determine the degree of pain and functional impairment; measurements were taken prior to and following induction and on each subsequent day.

Analysis of results using Freidman and Krushkall-Wallis H tests with *post hoc* Wilcoxon and Mann-Whitney U tests where appropriate, revealed significant differences ($p<0.05$) between Control and LIMIRT treatment groups for VAS, RANG and MPT scores (VAS at rest, day 2: $p=0.017$). Trends were repeatedly observed in favor of the treatment group with a significant treatment effect observed for MPT points 3-6 on day 2 ($p=0.032$); however, no significant differences between Treatment and Placebo groups were found. Results of the

current study are encouraging but provide only marginal support for the therapeutic effect of LIMIRT in the treatment of DOMS at these parameters.

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DEFOCALIZED LASER THERAPY 810 NM IN SPORT INJURIES

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A lot of lasers have been used in sport traumatology lesions, but their role is still under investigation. The Authors call this method Low Level Laser Therapy(LLLT).
We would like to investigate the clinical effects of laser 810 nm in the reduction of the recovery time of sport injuries. We used high level of energy, watts rather than milliwatts, reducing the duration of each application and the number of application for cycle.
We used a diode laser 810 nm, CW, spot/size 3 cm^2, and energy variable with the localisation and the nature of the injury. Establishing the exact dosage, we followed the clinical sings of phlogosis: when these sign were decreasing during the application, we continued till the patient had a less sensation of calor. We treated 56 patients both sex, 20-50 years old, selected following the same criteria: phlogosis sign present since two month,negative results with other two types of physical therapy, exclusion of surgical cases, exclusion of cases were pain is the only sign.
The results were divided in 4 groups for each pathology treated, following the parameters presence/absence of pain, phlogosis and functional limitation. The control were done compared similar cases no treated with laser, selected with random criteria.
We had an high percentage of positive results, in total. The reduction of irradiation time for each session and the reduction of the number of sessions necessary for to obtain the improvement of Clinical signs is very important. In opposite, the supposed negative effects of laser beam employed at high dosage of energy don't appear, because the skin and the other tissue don't present burns, if the radiation have a large spot (cm^2, rather than millimetres).
In conclusion, Defocalized Laser Therapy (DLT) seem efficacious as LLLT, in the treatment of sport injuries. DLT allows reducing the time of exposition for each session of therapy and the total number of sessions necessary to obtaining the results. In opposite, the interval between therapeutic dose and toxical dose is minus than with LLLT.

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LOW LEVEL LASER THERAPY IN THE TREATMENT OF OSTEOARTHROSIS OF JOINTS OF THE UPPER EXTREMITY: A MULTI CENTER, DOUBLE BLIND, PLACEBO CONTROLLED CLINICAL STUDY ON 154 PATIENTS

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Osteoarthrosis is the process of degeneration of joint cartilage with various different pathogenesis. Due to its functional importance and frequency among the population, we have decided to treat joints of the upper extremity in double blind, placebo controlled fashion. The leading symptoms of osteoarthrosis are stiffness, pain and joint deformation. Application of Low Level Laser Therapy (LLLT) in the treatment of osteoarthrosis is focussed on relieving of pain that would enable functional ability, which reduces and prevents stiffness and joint deformation. LLLT improves local cell metabolism, microcirculation of lymph and blood, too, what can significantly slow down osteoarthrotic degenerative process. This study has been performed on 154 patients in two specialized laser centers based at two countries. LLLT was applied as mono therapy with use of infrared diode lasers. Two application techniques were used: direct skin contact technique for treatment of trigger points and tender spots, and non-contact scanning technique for larger surface area. Follow up was conducted on the 6th, 12th and 18th month upon the initial LLLT. The results have demonstrated that the majority of patients have achieved more than 70% of pain relief and adequatly restored functional ability. Aside of relieving the major symptoms, advantages of LLLT of osteoarthrosis of upper extremity joints have demonstrated cost and time benefit, improved social activities, better quality of life, and the patients were free of side effects of drugs that is extremely important at chronic cases.

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THE ANALIZE OF THE EFFICENCY OF LOW POWER LASER TRETMENT IN SOME RHEUMATIC DISEASES

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Abstract

Usualy, in clinical practice it is dificult to appriciate the objectivity of the results. We treated with low power laser (LPL) 3 groups of patients.

Group A.: 126 patientes treated with infrared diode laser wavelength 780nm, 3.5 mW continous emmision, spot size 4mm;

Group B.: 142 patietes treated with infrared diode and He-Ne, wavelength 632nm, 2mW continous emmision, spot size 5mm;

Group C.: 98 patientes exposed to placebo laser;

For every patient the efficacy of the treatment was analized with an objective method, consisting in cutaneous ultrasound and also a subjective appreciation, using a pain scale.

To all patientes we analize the psychological profile.

Finally, following the Placebo exposure, we noticed that the patients' emotional tendencies and phsychological instability have a strong influence on the obtained good results.

We think that cutaneous ultrasound can be an objective method to appreciate the clinical results of LPL. The best results were optained when red and IR lasers are used togheter.

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LOW LEVEL LASER THERAPY OF ACNE AND SCARS APPLIED AS MONO THERAPY AND COMPLEMENTARY TREATMENT MODALITY TO TETRACYCLINE: A MULTI CENTER CLINICAL STUDY ON 80 PATIENTS WITH CONTROL GROUP

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The aim of this clinical study was to establish the efficacy and advantages of Low Level Laser

Therapy (LLLT) comparing to locally applied antibiotic tetracycline and the control, in the treatment of acne vulgaris and possible scars due to their esthetic and psychological aspects. The purpose was also to find a treatment modality to patients suffering from acne that previously developed side effects after applying tetracycline (e.g.,local redness, burning, itching, shedding, interactions to systemic antibiotics) or that have contraindications for its local use (e.g.,allergy, pregnancy, brest feeding). Current clinical study was conducted on 80 patients that were equally divided between two specialized laser centers where the same methods and techniques were applied. Acute and chronic patients were allocated in two groups used to evaluate efficacy of LLLT as follows: (1) LLLT vs. control (mono therapy), and (2) LLLT as a complementary treatment modality to tetracycline. LLLT was performed using non-contact technique on its two modalities, in all groups of patients: spot with infrared diode laser and scanning with Helium Neon and infrared. When LLLT was applied as mono therapy, the results have demonstrated significant relieve of all local clinical symptoms of acne. When LLLT was used as a complementary treatment to locally applied tetracycline, we have observed significant acceleration of the healing process, decrease in side effects and reduced occurrence of relapses. At all patients treated with LLLT, scars were significantly prevented or reduced.

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LOW LEVEL LASER THERAPY IN CHINA

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Shortly after the development of the first He-Ne laser in China in 1963, it was used in biomedical applications. Researchers found that He-Ne laser, when used at low power, affected the physiology and morphology of different tissues. Some of the effects observed included improved healing rates, changes in blood circulation, and decreases in the anti-inflammatory responses. In the early 1970's, quartz optical fiber technology was coupled with the He-Ne laser; this advance allowed light to be used instead of a needle in traditional acupuncture method. For more than a decade, He-Ne low-level laser therapy(LLLT) has been used in China to treat more than 100 types of diseases and trauma. For this report about 16,900 cases between 1985 and 1994, which received He-Ne LLLT at 203 hospitals in China, were reviewed. These patients were seen in departments of dermatology, internal medicine, neurology, otolaryngology, anesthesiology, gynecology, and obstetrics. Some of diseases treated include arthritis, hypertension, asthma, dermatitis, paronychia, eczema, zoster, sciatica, facial paralysis, allergic rhinitis, pharyngitis, parotitis, chronic laryngitis and anesthesia for tooth extraction. The range of treatment parameters used included: power from 2-10 mW, irradiation on the lesion or acupuncture points for 2-5 minutes per point, 2 to 4 points each time, normally 10 times a course. In recent years, He-Ne LLLT was extended to intravascular applications by optical fiber for stroke and angioplasty, and diode low level laser has begun to be used for the various diseases in dermatology and urology. The use of He-Ne LLLT and the positive outcome of therapy on thousands of Chinese patients provides a convincing body of data for the potential development and use of LLLT in other countries.

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LOW LEVEL LASER THERAPY IN THE TREATMENT OF CERVICAL SYNDROME: A MULTI CENTER, DOUBLE BLIND, PLACEBO CONTROLLED CLINICAL STUDY ON 128 PATIENTS.

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The purpose of this clinical study was to assess the efficacy of Low Level Laser Therapy (LLLT) in the treatment of cervical syndrome due to more frequent appearance of this disorder even in school age (i.e., computer generation). Current clinical study was performed on 128 patients suffering from acute and chronic type of bilateral and unilateral cervical syndrome caused by cervical soft tissue trauma. Exclusion criteria were as follows: congenital and trauma-related anatomic deformations of cervical spine, fracture of cervical spine vertebrae and injury of corresponding spinal nerves. LLLT was applied as mono therapy using two application techniques: direct skin contact technique in the treatment of trigger points, and non-contact scanning technique for larger surface area, both with use of infrared diode laser. Outcome measurements were performed using objective and subjective evaluation criteria, and finally postponed to statistical analysis. The results have demonstrated significant reduce in all clinical symptoms comparing to placebo. Laser application on trigger points interrupts the vicious circle of the origin of the pain and its development and brings a normal physiological condition back to the tissue. Negative side effects and uncontrolled use of drugs especially in chronic cases have been reduced or even eliminated with use of LLLT. We have observed that patients treated with LLLT developed better quality of life with significantly improved sleeping pattern, they were professionally more efficient and have had cost and time benefit overall.

CARDIOVASCULAR

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TRANSMYOCARDIAL LASER REVASCULARIZATION IMPROVES MYOCARDIAL BLOOD FLOW IN CHRONIC ISCHEMIA

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PURPOSE: Magnetic Resonance (MR) tagging technique can quantify and perhaps explain some mechanisms of TMLR. The purpose of this study was to evaluate resting functional response of myocardium following TMLR by MRI techniques.

METHODS: Two groups of swine were studied 1 and 9 weeks following intervention. Group I (n=5) had left circumflex artery (LCX) embolization. Group II (n=6) had LCX embolization and TMLR 1 week post embolization. Wall motion changes were studied with MR tagging at 1 and 9 weeks and compared to myocardial blood flow

(MBF{ml/min/gm tissue}) in the LCX region, peri-ischemic zone, and remote anterior area in the zone perfused by the left anterior descending artery (LAD).

RESULTS: MBF increased significantly after TMLR in Group II as indicated in Table I. In group I a significant increase in circumferential/radial transverse shear was noted in the LCX after 9 weeks, indicating functional decline.

	LCX	LAD
Group I	0.22±0.4	0.69±0.11
Group II	0.59±0.16 p<0.4	1.18±0.04 p<0.003

CONCLUSION: For the first time salvage of contractility at rest was demonstrated following TMLR by using MR tagging techniques. Improvements were noted in the ischemic and remote zones. MR tagging allows for quantitative and precise assessment of function following TMLR. Functional recovery of the myocardium and improved MBF indicates TMLR probably does protect the ischemic heart.

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COMPARISON BETWEEN CO2 AND HOLMIUM LASERS FOR TRANSMYOCARDIAL REVASCULARIZATION: ALLIED HEALTH PERSPECTIVE

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Transmyocardial Revascularization (TMR) is a procedure in which a CO2 or Holmium laser is used to place small channels into oxygen-deprived heart muscle. Currently, Abbott Northwestern Hospital has a high-powered CO2 laser and a fiber optic delivered Holmium laser. After participating in the Phase III clinical study for Holmium laser TMR, our first post-clearance procedure was performed in February of 1999. This report compares the two laser systems on the following factors: (1) operation (2) safety considerations (3) costs and (4) reimbursement, and discusses our experience in the initial series of cases, including some technical data.

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OPTICAL COHERENCE TOMOGRAPHY GUIDED TRANSMYOCARDIAL LASER REVASCULARIZATION

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Transmyocardial laser revascularization (TMR) can result in ventricular perforation when laser energy is delivered percutaneously by catheters guided by fluoroscopy. Optical coherence tomography (OCT) was evaluated in this study as a means to guide catheter based laser ablation of porcine myocardium. OCT was performed via a 200 μm optical fiber using a 1310 nm diode laser (IntraLuminal Therapeutics, Inc.). The OCT fiber was incorporated into a 1.8 mm diameter laser catheter with a circumferential array of optical fibers delivering 308 nm pulsed excimer laser energy (Spectronetics) at 50 mJ per pulse at a rep rate of 30 Hz. Laser energy was applied with the laser catheter perpendicular to the endocardial surface and myocardium ablated until real-time OCT detected the proximity of the epicardium. OCT was able to distinguish the epicardium between 100 and 1000 μm in each case of laser ablated myocardial channels via a well defined reflectance spectra from the epicardium which provided a signal to halt laser ablation prior to perforation. The signal to noise ratio for epicardium detection was as high as 20 dB. Intentional perforation in some cases could be easily confirmed and predicted with an easily distinguishable OCT spectra. We conclude that OCT is a feasible, safe and easily incorporated means of guiding catheter based percutaneous TMR and may reduce the incidence of ventricular perforations in this new therapy.

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PROANGIOGENIC EFFECT OF LOW POWER LASER IRRADIATION IN VITRO

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Numerous reports suggest that Low Power Laser Light (LPRLL) is capable of affecting cellular processes in the absence of significant thermal effect. The objective of the present study was to determine the effect of LPRLL on VEGF secretion and proliferation of Human endothelial cells (EC) and smooth muscle cells (SMC) in vitro. All cell cultures were irradiated with single dose (5mW) of He-Ne continuos wave laser (632 nm) for 1, 3, 5, 10 and 20 min. Assessment of effect on proliferation was performed utilizing Alamar blue assay. VEGF secretion by SMC was studied with sandwich enzyme technique. It was found that1) enhancement of EC growth can be obtained with LPRLL and it increased to 180% at 10 minutes of LPRLL exposition, 2) The VEGF secretion by SMC was observed at 3, 5, 10 and 20 min dose and was maximal at 5 and 10 min Laser exposure (167.5% and 156.8% respectively) These data may have significant importance leading to the establishment of new methods for photoangiogenesis.

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LOW-POWER RED LASER LIGHT PROMOTES PROLIFERATION OF CARDIOMYOCYTES IN VITRO

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We hypothesized that LPRLL may contribute to tissue repair following myocardial injury. Accordingly, we studied the effect of LPRLL irradiation in the fetal cardiomyocytes in vitro. All cell cultures were irradiated with a single dose (5mW) using a He-Ne continuos wave laser (630 nm) for 5, 10, 15 and 20 min. Assessment of effect was performed after 18 hours following irradiation. Effect of LPRLL on new DNA synthesis was studied by 3H-Thymdine incorporation assay; VEGF and TGF-β expression was studied by RT-PCR.

	5 min	10 min	15 min	20 min
TGF-β	0.25±0.25	0.71±017	0.80±0.05	0.38±0.11
VEGF	0.12±0.08	1.10±0.12	1.31±0.80	0.20±0.32

It was found that (1) increased cardiomyocyte proliferation (10-60%) can be obtained with LPRLL and this effect is dose dependent; (2) there is a significant dose dependent increase in VEGF and TGF- β mRNA expression. This preliminary data suggests that LPRLL

induces increased proliferation of Cardiomyocytes and increases the production of VEGF and TGF-β in vitro. Further in vivo studies are warranted, hence this data may have significant importance leading to the establishment of the new methods for myocardial photo-regulation and photo-angiogenesis.

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SMALL EXCIMER LASER ANGIOPLASTY CATHETER FOR FIBROCALCIFIC TISSUE PENETRATION

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Background and Objective: Excimer laser angioplasty catheters (ELAC) have historically had difficulty penetrating calcified lesions. A new fiberoptic catheter was developed to improve calcified atherosclerotic plaque penetration. The new catheter features a 0.9mm outer diameter, high density optical fiber arrangement at the tip, and laser parameters up to 80mJ/mm^{2}fluence and 80Hz pulse repetition rate. The object of this study is to determine the penetration performance of the new catheter in various tissue types, measure the associated thermal and mechanical effects, and compare to commercially available ELAC. **Materials and Methods:** The rate of device penetration through soft and hard tissue was measured as pulsed ultraviolet light from a 308nm XeCl excimer laser was applied. Independent variables were fluence (60/80mJ/mm^2) and laser pulse repetition rate (40/80 Hz). Routine histological processing was performed in all ablated samples. The extent of thermal and mechanical effects was measured under polarized light microscopy. **Results:** The 0.9mm catheter demonstrated increased penetration rates through all tissue types when compared to commercially available catheters. Penetration rates of 824±368 and 592±376 microns per second were obtained in soft and hard tissue respectively. The measured mechanical and thermal effects were similar to commercially available catheters. Maximum extent of thermal effects was 78±20 microns for soft tissue and 382±75 microns for hard tissue. Maximum extent of mechanical effects was 377±272 microns for soft tissue and 597±148 microns for hard tissue. Improvement in penetration could be attained by increasing fluence and pulse repetition rate. Increase in average thermal and mechanical effects corresponded to increased fluence and repetition rates. **Conclusions:** In-vitro testing of the 0.9mm catheter has demonstrated increased penetration in hard tissue while maintaining associated thermal and mechanical effects within accepted ranges. Study of the new catheter to determine effectiveness in penetrating fibrocalcific atheroma in a clinical setting is warranted.

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EXCIMER LASER EFFECT ON PLATELET AGGREGATION KINETICS

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Purpose: Platelets, a major constituent of thrombus, play a crucial role in pathogenesis of acute ischemic coronary syndromes and in complications during percutaneous interventions. The effect of excimer laser emission on platelets within thrombi is unknown. ***Methods:*** Blood samples were obtained by atraumatic venipuncture of twenty-two normal volunteers. All samples were anticoagulated with 3.8% sodium citrate. The samples were immediately divided into 2 ml fractions. Samples were exposed to several levels (0,30, 45, 60 mJ/mm^2; 25Hz) of excimer laser energy at a 308nm wavelength, 135nsec pulse duration, 200mJ/pulse (CVX-300, Spectranetics Inc., Colorado Springs, CO). All exposures were done in triplicate. Samples were then tested for ADP and collagen induced whole blood aggregation and for platelet contractile force (PCF) development. ***Results:*** Exposure to excimer laser energy produced dose dependent suppression of platelet aggregation and force development. Platelet contractile forces declined from 34,500±3700 to 27,800±2700 dynes as laser energy increased from 0 to 60 mJ/mm^2 ($p<0.03$). ADP aggregation decreased from 7.9±1.1 to 4.0±0.8 Ohms ($p<0.001$) to 3.1±0.0 Ohms ($p<0.03$) and to 2.0±0.6 Ohms ($p<0.03$) as the laser energy increased from 0 to 30 to 45 to 60 mJ/mm^2, respectively. Collagen induced aggregation decreased from 21.2±1.5 Ohms to 15.3±1.3 Ohms ($p<0.001$) and to 11.4±1.2 Ohms ($p<0.001$) and to 9.6±1.0 Ohms ($p<0.01$), in response to the same incremental range of laser energy. ***Conclusion:*** Aggregation kinetics are altered in platelets exposed to excimer laser energy. This phenomenon is manifested by decreased platelet aggregation and reduction in platelet force development capability. The response is dose dependent and most pronounced at higher energy levels such as 60mJ/mm^2.

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APPLICATION OF AN ERBIUM LASER FOR TRANSMYOCARDIAL REVASCULARIZATION

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Most transmyocardial laser revascularization studies have used infrared lasers, specifically carbon dioxide and holmium:YAG, to create the channels. Despite the presumed efficacy of such wavelengths, other infrared lasers have received little or no consideration even though erbium lasers have been shown to have good ability to cut both hard and soft tissues. Therefore, a fiber delivered erbium:YAG laser (2.94μm; Pharos Optics, Los Angeles) was evaluated for TMR. In vitro study (3-9W, 20Hz) revealed that mechanical damage to tissue surrounding the channels (indicated by dissections) extended for ≈1mm and exceeded the amount of thermal damage (indicated by loss of muscle birefringence viewed with polarized light) by a ratio of 3:1. This injury was considered too extensive and so the power was reduced for in vivo use. Six channels were made in each of 9 rat hearts. At pulse energies >100mJ pulsatile bleeding from the channels could not be controlled. Thus, the energy was further decreased to 80 and 40mJ at 10 or 20Hz. However, rats with channels made at ≥0.8W died within hours of TMR. The 5 surviving rats (0.4W) were subjected to coronary artery occlusion 1-2 months after TMR. All of these hearts had evidence of cardiac protection, as revealed by triphenyltetrazolium staining to detect viable myocardium. Moreover, open channels with direct connection to the left ventricular cavity were identified. These findings are consistent with blood flow through the channels. In conclusion, the benefit of erbium laser TMR appears, from this pilot study, to be highly dependent upon pulse energy and success was achieved at powers much lower than expected based on previous infrared laser trials.